

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims

1. (Previously presented) An avian cell line immortalized by non-viral transfection with a combination of viral and/or cellular genes (gene (s)), at least one first gene affecting the function of the retinoblastoma protein by mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors and at least one second gene affecting the p53 protein or a family member thereof, wherein the second gene is a viral gene coding for a protein preventing induction of growth arrest and apoptosis by p53, or is a cellular gene preventing growth arrest and apoptosis by p53.
2. (Original) The avian cell line of claim 1, wherein the first gene overcomes G1 checkpoint control and the second gene prevents apoptosis induced by the first gene.
3. (Currently amended) The avian cell line of claim 1 ~~or~~ 2, wherein
 - (i) the cell line is derived from embryonic or hatched chicken, duck, goose or quail, preferably from chicken or duck; and/or
 - (ii) the cells subjected to immortalization are primary cells including fibroblasts, cells from isolated body segments (somites) or separated individual organs including neuronal, brain, retina, kidney, liver, heart, muscle and extraembryonic tissues and membranes protecting the embryo; and/or

(iii) the immortalization by non-viral transfection, includes, but is not limited to, liposome and dendrimer-mediated transfection and electroporation; and/or

(iv) the first gene is a viral gene mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors such as an adenovirus E1A gene from mastadenoviruses, preferably from mastadenoviruses of group C, an E7 gene of papillomaviruses, preferably from low-risk human papilloma virus (HPV) (such as HPV 1, HPV6 and HPV11, but not HPV16 and HPV18), an orf 22 gene of avian adenoviruses, E43 open reading frames from ovine attadenovirus, etc.; or is a cellular gene mediating disruption of complexes between retinoblastoma proteins and E2F transcription factors such as Cyclins D1, D2 or D3, a mutated CDK4 not susceptible to inactivation by p16INK4a, etc.; and or

(v) the second gene is a viral gene coding for a protein preventing induction of growth arrest and apoptosis by p53 such as the adenovirus E1B55K protein of all groups, GAM-1 of CELO, the E6 protein of papillomaviruses, preferably those of the low-risk HPV (such as HPV 1, HPV6 and HPV11, but not HPV16 and HPV18), or is a cellular gene preventing growth arrest and apoptosis by p53 such as mdm2, etc.; and or

(vi) the first gene and second gene are separated spatially by heterologous sequences or are located on different nucleic acid segments or plasmids.

4. (Previously presented) The cell line of claim 3, which is immortalized with

(i) the E1A (first gene) and E1B (second gene) region of an adenovirus from the genus Mastadenovirus, preferably said E1A and E1B region is derived from adenovirus 5, more preferably said E1A regions have the sequence of bp 1193 to2309 of SEQ ID NO:7 or

the sequence complementary to bp 4230 to 3113 of SEQ ID NO:9, and said E1B regions have the sequence of bp 1145 to 3007 of SEQ ID NO:8 or the sequence complementary to bp 2345 to 550 of SEQ ID NO:9; and or

(ii) the genes orf22 (first gene) and GAM-1 (second gene) from an adenovirus, preferably from the genus aviadenovirus CELO, which preferably have the sequence represented by the sequence complementary to bp 1252 to 635 of SEQ ID NO:10, and the sequence complementary to bp 3138 to 2290 of SEQ ID NO:10, respectively; and/or

(iii) combinations of nucleic acids encoding E1A and/or E1B with GAM-1 and/or Orf22 as defined in (i) and (ii) above.

5. (Currently amended) The cell line according to claim 1 ~~anyone of claims 1 to 4~~, which

(i) additionally carries non-natural functional sequences including, but not limited to, transgenes such as genes complementing deficient viruses (e.g. EBNA1, etc.), promoters (e.g. PGK-, EF1.alpha-, CMV-promoter, tk-promoter, etc.), enhancers (e.g. RSV-LTR), selection markers such as neomycin-resistance, puromycin-resistance, etc.; and/or

(ii) is suitable for production of biologicals or viruses including vaccine strains and recombinant viral vectors.

6. (Currently amended) The cell line according to claim 1 ~~anyone of claims 1 to 5~~, which

(i) is free of reverse transcriptase activity; and/or

(ii) is derived from immortalization of a primary cell originating from duck embryos or hatched ducks; and/or

(iii) is derived from extraembryonic membrane; and/or

(iv) is cultivated in a chemically defined medium which is preferably free of animal serum.

7. (Currently amended) The cell line of claim 1 ~~claims 1 or 6~~, which is avian cell line 12A07-A10 (DSM ACC2695).

8. (Currently amended) A method for preparing a cell line according to claim 1 ~~anyone of claims 1 to 7~~, which comprises transforming/transfecting a starting cell with the first and second gene.

9. (Previously presented) The method of claim 8 which comprises non-viral transfection of the starting cell.

10. (Currently amended) Use of the cell line according to claim 1 ~~anyone of claims 1 to 7~~ for the production of biologicals or viruses.

11. (Currently amended) A method for producing viruses which comprises

(i) contacting said viruses with a cell line according to claim 1 ~~any one of claims 1 to 7~~; and/or

(ii) cultivating said viruses on said cell line.

12. (Previously presented) The method of claim 11 for producing a pox virus, preferably strain MVA, in a duck cell line, preferably a cell line originating from duck somites or duck neuronal tissue, even more preferred from duck retina.

13. (Currently amended) A method for producing recombinant proteins which comprises

(i) introducing a gene coding for a recombinant protein, operably linked to a promoter, into a cell line according to claim 1 ~~any of claims 1 to 7~~,

(ii) cultivating said modified cell line and

(iii) harvesting the recombinant protein.